Effect of milk feeding strategy and lactic acid probiotics on growth and behavior of dairy calves fed using an automated feeding system¹

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ABSTRACT: Automated milk feeders offer flexibility to feed calves high milk allowances, to change the daily quantity of milk offered, and also to dispense additives like probiotics on an individual basis. Our objectives were to test the effects of 2 milk feeding protocols and a lactic acid bacterium probiotic on performance and behavior in calves. Heifer dairy calves ($n = 96$) were enrolled at birth in a 2×2 factorial study design comparing feeding (1) 2 milk feeding protocols and (2) a lactic acid bacterium-based probiotic program, or a placebo, using automated milk feeders. The early milk feeding strategy (EM) offered a maximum of 11 L/d on day 1 and a peak maximum allowance of 15 L/d on day 21. The late milk feeding strategy (LM) offered a maximum of 7 L/d on day 1 and increased slowly to its peak at 13 L/d on day 28. Both feeding strategies gradually weaned the calves after peak milk allowance until complete weaning at day 53, offering a total of 543 liters of milk. Probiotics or placebo were fed orally in a gel once after colostrum, and twice daily in the milk until weaning. Water and calf starter were provided *ad libitum*. The experimental period was divided into 3 periods: from day 1 on the automated feeder to day 28 (Period 1), from day 29 to day 53

(Period 2), and the week post-weaning (Period 3). For Period 1, the average daily gain (ADG) of the probiotic group was greater than that of the placebo group (0.84 ± 0.10 kg/d vs. 0.74 ± 0.10 kg/d, respectively), but was not different between milk feeding strategies. For Period 2, ADG was not affected by probiotic or milk feeding strategies. For Period 3, ADG was greater for EM compared to LM (1.27 ± 0.10 kg/d vs. 1.02 ± 0.10 kg/d, respectively), but not between probiotic and placebo groups. During the whole experimental period, LM calves consumed significantly more milk than the EM calves (431.84 ± 33.0 liters vs. 378.64 ± 34.2 liters, respectively). During Period 3, probiotics affected the frequency of visits to the calf starter feed bunk (37.72 ± 2.8 vs. 23.27 ± 2.8 visits per day for probiotic and placebo groups, respectively), but did not affect total time spent at the feed bunk. The supplementation of a lactic acid-based probiotic improved ADG during early life and altered some aspects of the feeding behavior of dairy calves. Calves receiving an early accelerated milk allowance had improved growth during post-weaning and consumed less milk in total, which may indicate better use of solid feed.

Key words: accelerated feeding program, animal welfare, group-housing nutrition, probiotic

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INTRODUCTION

Raising dairy calves in a group setting is becoming more common in dairy farms around the world; in a recent survey, 15% of dairy farms in the United States reported using this system (U.S. Department of Agriculture [USDA], 2016).

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A group dynamic offers many benefits for calves (see review by [Costa et al., 2016](#)), and when used in combination with computer-controlled automated feeding systems, it provides the farm with the possibility to use individualized milk feeding strategies and to record data such as individual milk intake, drinking speed, and feeder visits ([Knauer et al., 2017](#)). Individualized milk feeding strategies involves changing the quantity of daily milk offered; research in automated milk feeding systems has primarily focused on individualizing weaning strategies (see review by [Khan et al., 2011](#)); however changing the daily milk allowance offered to the calf in early life is a large opportunity for this feeding system. For example, in nature during the first few weeks of life, calves will suckle the dam in several small meals, and suckling time decreases as the calf ages ([Fröberg and Lidfors, 2009](#)); this meal pattern is similar to when calves are offered milk *ad libitum* from automated milk feeders (see review by [Miller-Cushon and DeVries, 2015](#)).

Feeding calves high amounts of milk may be discouraged since little grain is consumed prior to weaning, even when using a gradual (step-down) weaning method ([Rosenberger et al., 2017](#)). Since rumen development is associated with grain intake (see review by [Kertz et al., 2017](#)), it is important to encourage early attendance to the calf starter feed bunk. Moreover, many producers are interested in the advantages of feeding higher milk allowances using automated feeding systems without increasing labor ([Hotzel et al., 2014](#); [Medrano-Galarza et al., 2016](#)). In individual housing, calves fed a higher quantity of milk replacer at a denser crude protein level (28% during the first month of life compared to a standard 21.5% milk replacer offered until 37 d of life) led to maintained growth advantages accrued prior to weaning, and an earlier puberty age ([Davis Rincker et al., 2011](#)). In addition, feeding higher milk allowances to calves may also be associated with higher milk production in the first lactation ([Gelsinger et al., 2016](#)). Therefore, it is of interest to determine if offering a milk feeding strategy which offers peak levels of milk early in life (21 feeder days to encourage early calf starter visits, or 28 feeder days, to provide more time to adjust to the feeder when first enrolled) can offer the benefits of feeding higher milk throughout the entire pre-weaning period without compromising solid feeding behavior.

Another criticism of feeding high milk allowances to calves is that feed efficiency on a mega calorie per kilogram gained is reduced, particularly post-weaning ([Dennis et al., 2017](#)). One potential

way to improve growth in calves is through the use of probiotics. Probiotics can stabilize microflora populations within the small intestine, outcompete deleterious pathogens, and enhance intestinal barrier function; this phenomenon is called competitive exclusion ([Ohland and MacNaughton, 2010](#)). When directly fed to calves at restricted milk allowances in an automated feeding system, lactic acid microbiota increased milk intake, inoculated the calf gut, and increased the lactobacillus-to-coliform ratio of feces, thereby improving average daily gain (ADG; [Soto et al., 2014](#)). Limit-fed calves may also benefit from probiotics when under environmental stress. Indeed, in calves with high disease prevalence, the yeast *Saccharomyces cerevisiae* increased papillae development, improved feed efficiency, and improved ADG ([Alugongo et al., 2017](#)). Similarly, a meta-analysis concluded that calves fed viable, lactic acid probiotics showed growth advantages, though these benefits only applied to when calves were fed milk replacer, and the included studies limit-fed the calves to no more than 6 L/d ([Frizzo et al., 2011](#)). It is unknown if probiotics will similarly affect performance and feeding behaviors in calves fed higher allowances of milk.

The objectives of this study were to measure the effects of 2 milk feeding protocols which changed the daily milk offered to calves (milk feeding strategy) and a lactic acid bacterium-based probiotic program on performance, feeding, and lying behavior in calves. For the milk feeding strategies, we manipulated the timing of peak milk allowance to either 21 feeder days (early) or 28 feeder days (late). For the lactic acid bacteria-based probiotic program, we were interested in determining the effect of feeding a probiotic regime from birth until weaning on calves fed high amounts of milk early in life. We hypothesized that calves receiving an early peak milk allowance would present higher ADG, since a subsequent early reduction in milk would encourage uptake of solid feed.

MATERIALS AND METHODS

The study was conducted from February to December 2014 at a commercial dairy farm in Dane, WI, USA. The University of Wisconsin-Madison Animal Care and Use Committee approved all procedures for this research protocol (protocol number: A01554-0-11-13).

Study Design and Treatments

Holstein heifer calves ($n = 96$; late milk placebo [$n = 23$], late milk probiotics [$n = 25$], early milk placebo [$n = 21$], and early milk probiotics [$n = 27$])

were randomly enrolled in balanced blocks of 20 to reflect maximum pen size in a 2×2 factorial design at birth. Power analysis (following Hintze, 2008) calculations found that this sample was adequate to detect a change of 0.10 kg/d ADG between treatments with 90% power and 0.05 α . Estimates of variation for ADG were based on the reported values in Hill et al. (2010). Calves were assigned to one of 2 milk feeding strategies upon enrollment: either an early peak milk feeding strategy (EM) or a late peak milk feeding strategy (LM), both milk feeding strategies differed in timing of peak milk offered, timing of milk decline, and in slope of milk offered (Figure 1). Both programs offered 543 liters of unsaleable pasteurized whole milk, and gradually weaned calves until complete weaning at 54 d. The calculated amount of milk required to achieve 0.80 kg ADG was 543 liters, while ensuring an appropriate transition to solid feed during weaning (National Research Council [NRC], 2001). The EM offered a maximum allowance of 11 L/d beginning on day 1, and the milk allowance was divided automatically by the automated feeder so that the daily maximum milk allowance increased in increments to a peak of 15 L/d on day 21. The LM offered a maximum allowance of 7 L/d beginning on day 1, and the daily milk allowance increased incrementally until it peaked at 13 L/d on day 28. Calves remained on the study for 1 wk post-weaning.

Calves were also enrolled on a viable, multi-strain, lactic acid bacteria-based probiotic program or a placebo which was added to the milk and fed to the calves from birth until weaning was complete. The 3 lactic acid bacteria-based probiotic products were designed to be used as a collective treatment and were as follows: one 10 cc paste of

a probiotic gel (Colostrum Booster Gel, DeLaval, Waunakee, WI, USA) fed orally after the delivery of 4 liters of colostrum; a 5 cc scoop of a probiotic supplement (Feedtech Calf Probiotic Supplement, DeLaval, Waunakee, WI, USA) mixed in the milk and fed twice per day from birth until enrollment in the automated feeding system; and a 1.75 g probiotic supplement (Feedtech DFM Supplement, DeLaval, Waunakee, WI, USA) mixed twice per day automatically in the automated feeding system while calves were enrolled on the automated feeder. The placebo was a soybean-based product and appeared visually identical. Farm and research staff were blind to treatment assignment. The following strains were present in all 3 probiotic products: *Enterococcus faecium* M74, *Lactobacillus acidophilus*, *Lactobacillus casei*, *S. cerevisiae*, *Bifidobacterium bifidum*, and *Lactococcus lactis*.

Animals, Diet, and Management

All calves were removed from the dam within 2 h and fed colostrum via an esophageal feeder if colostrum quality was higher than 22.4% BRIX; otherwise, the farm staff fed 1 dose of colostrum replacer, offering at least 50 g of IgG (Land O' Lakes Colostrum Replacer, Land O' Lakes, Shoreview, MN, USA). After calves received colostrum, they were transported to a naturally ventilated barn equipped with 2 positive pressure tubes which was divided into 4 group pens, and contained individual housing (Calf-Tel Pens, Hampel Corp., Germantown, WI, USA) for newborn calves. Caustic paste was applied for dehorning at 24 h of age (Dehorning Paste, H. W. Naylor Co., Morris, NY). While individually housed, calves were fed 2 liters of milk replacer (Medicated Amplifier Milk Replacer, Land O' Lakes, Shoreview, MN, USA) twice daily by bottle until feeder enrollment at 7 ± 2 d of age. All pens in the automated feeding system were managed as a dynamic group (or continuous flow) with age and stocking density influencing when calves were moved to the next group. All calves received 16 g of electrolytes in 2 liters of solution (Land O' Lakes Electrolytes System, Land O' Lakes, Shoreview, MN, USA) from farm staff as preventative therapy at 10 d of age, and as needed for treatment of additional diarrhea symptoms.

This barn on the farm had 4 group pens which were managed as continuous flow based on age of the calves and stocking density of the pens. Since study treatments were randomly assigned by birth date, pens were always balanced by treatment. The first 3 pens had 1 nipple per pen with access to

Figure 1. Daily milk (L) allowance up to 53 d offered to Holstein heifers ($n = 96$) fed either a milk feeding strategy that peak at 21 or 28 d¹ and lactic acid probiotic, or placebo². ¹Treatments: EM = early milk feeding strategy feeder day 1 offers 11 L/d and milk peaks at 15 L/d at 21 feeder days; LM = late milk feeding strategy feeder day 1 offers 7 L/d and milk peaks at 13 L/d at 28 feeder days. ²Probiotic = active lactic acid strain probiotic fed to calves 2×/d while on the feeder.

the automated calf feeder. The fourth pen was for calves after weaning and did not have access to an automated feeder. Upon feeder enrollment at 7 ± 2 d, calves were moved to a pen (11.28×3.05 m) that was bedded with corn stalks and contained 4 to 6 other calves for training to access the automatic milk feeder. Water and calf starter (Ampli-calf Starter, Purina Animal Nutrition, Arden Hills, MN, USA), contained in the same pen, were provided in all pens ad libitum. Calf starter was made available in an open bunk (1.52×0.31 m) positioned adjacent to the water in each pen. The calf starter contained 60 g/ton of Lasalocid and a minimum 20% crude protein and 2% crude fat. Once calves were trained to access the automatic milk feeder, and after consuming 4 L/d independently, they were moved into a different pen (13.72×5.49 m) that was bedded the same, also contained water and calf starter, and contained 16 to 20 calves (4.18 to 4.80 m² per calf). After the calves had received milk for 30 d, they were moved to a third pen that was identical to the previous pens. After being weaned from the feeder for 4 d (day 57), calves were moved to the fourth pen. Calves were fed unsaleable pasteurized whole milk 3×/d from the farm's pen of fresh cows. Milk was collected in buckets, batch pasteurized (CMP 2000, Darlington Dairy Supply Co., Darlington, WI) at 63 °C for 30 min then automatically cooled, and immediately moved to a holding tank (cleaned daily using acid detergent used in the parlor) adjacent to the automated calf feeder (DeLaval Calf Feeder CF1000+, DeLaval, Waunakee, WI).

Milk Intake and Behavior at Milk Feeder

The experimental period was divided into 3 periods: from the day of enrollment on the feeder (7 ± 2 d) to day 28 (Period 1), from day 29 to day 53 (Period 2), and from the week post-weaning at 60 ± 2 d to a final measurement age at 67 ± 2 d (Period 3).

Daily milk intake (L/d) and average drinking speed (mL/min) were recorded by the milk feeder and summarized for each of these periods. Daily milk allowance was limited, offering a maximum meal size at a given time. For feeder day 1 to feeder day 8, milk meal size offered 0.5 liter and a maximum of 2.5 liters. For feeder day 9 to feeder day 53, milk meal size offered 1.0 liter and a maximum of 2.5 liters. For assessing milk intake, 8 frames were chosen to best divide the data into week (every 6.7 d) on the feeder when milk feeding strategies changed.

At each herd visit, a milk sample from the milk tank was collected after 10 min of mixing after

filling, and total solid contents were determined for the unsaleable pasteurized whole milk that was offered to the calves. Milk samples were assessed for nutrient content at a lab (AgSource Laboratories, Verona, WI, USA) and averaged % dry matter (DM) 13 ± 0.1 , % crude protein 3.1 ± 0.1 , and % fat 3.9 ± 0.1 . In order to ensure milk fed to calves was adequate total solids, weekly total solids were also measured using a BRUX refractometer (VEE GEE Laboratories, Kirkland, WA, USA) and were calculated using the equation from [Moore et al. \(2009\)](#). BRUX total milk solids were $13 \pm 1\%$. Per protocol by the herd veterinarian, and sterile milk samples were also collected weekly from the nipple in the pen that contained the youngest calves; the samples were collected after an automated rinse cycle in the feeder to assess milk cleanliness. Milk cleanliness ($n = 34$) total plate count (TPC) was 100,000 cfu/mL (2,800 to 1,800,000 cfu/mL) (median; inter-quartile range).

Two temperature monitors (Hobo, Onset Computer Corp., Bourne, MA, USA), positioned on opposite sides of the barn, recorded ambient temperature for the duration of the experiment. Ambient temperatures averaged 13.0 ± 9.7 °C (mean $\pm$ SD) and ranged from -16.9 °C to 33 °C throughout the study period. During the winter (February through April), the temperature range was -16.9 °C to 21.9 °C with a mean 5.4 ± 7.9 °C ($\pm$ SD). During the summer (May through August), temperatures ranged from 4.1 °C to 32.6 °C with a mean 5.4 ± 19.9 °C. During the fall (September through November), the temperature range was -10.9 °C to 33.0 °C with a mean 9.7 ± 11.9 °C.

Data Collection Measures

Calf vigor and passive transfer. All farm staff received training from a licensed veterinarian for dystocia scoring, calf vigor assessment, and colostrum administration. The farm staff were also trained to give every newborn calf a vitality score, which is a combination of a dystocia score and a calf vigor score ([Murray et al., 2015](#)). Briefly, the calf vigor score included scoring calves for response to a stimulus (straw), gum color, respiration rate, heart rate, and time to stand ([Murray et al., 2015](#)).

To assess for passive transfer of immunity, at mean 3 ± 1 d of age, a 5 mL blood sample was collected into tubes not containing anti-coagulant via jugular venipuncture, placed on ice, and centrifuged at $6,000 \times g$ for 15 min at 0 °C. The blood serum was pipetted off and analyzed using a

BRIX optical refractometer (VEE GEE Scientific, Kirkland, WA) at a cutoff of 8.4% for failure of passive transfer (Deelen et al., 2014).

Growth and health measures. An initial baseline weight recorded electronically (Amston Scale Company Inc., Amston, CT) and a wither height using a measuring Stick (Nasco Measuring Stick, Nasco, Fort Atkinson, WI) were taken for every calf at 3 ± 1 d of age. Then, every Tuesday and Thursday until 2 test dates post-weaning, a weight, height, and a health exam for Bovine Respiratory Disease (BRD), rectal temperature, umbilical status, and diarrhea were recorded.

Health exams were performed according to the procedure of the Wisconsin Calf Respiratory Scoring Chart (Poulsen and McGuirk, 2009). Briefly, BRD was defined as a total score of 5 or greater including presence of nasal discharge, degree of coughing, temperature status, and the highest ear, or eye score. A case duration was defined as 10 d, and antibiotics were administered on the first exam by farm staff at the first presence of these signs (Heins et al., 2014). Farm staff re-treated the calves after they identified a second case. For third cases, the veterinarian assessed the calf and classified her as failure to cure (chronic) after 10 d of a second antibiotic treatment. Depending on severity and first or repeated case, farm staff administered antibiotics following the recommendation of the herd veterinarian.

Every health exam also included a palpation of the umbilical site (per herd veterinarian), and was graded as a score of zero (closed), 1 (open, normal), or 2 (swelling, clouded discharge). Farm staff treated all positive umbilical site palpations (score of 2) with penicillin for 10 d. Incidence of positive umbilical site palpation was 5% (5/96).

Fecal scores were also assessed by rectal stimulation of each calf on each exam. Fecal score (per herd veterinarian) was considered normal if the fluidity ranged from solid to the fluidity of pancake batter. If the fluidity was that of orange juice, a case of diarrhea was diagnosed (Larson et al., 1977). Case duration was the date of the health exam. Calves did not receive antibiotics for diarrhea.

Originally 104 calves were enrolled, but 8 calves died and were excluded from analysis due to early death (less than 10 d old). Mortality by study treatment was: LM placebo: $n = 2$, LM probiotics: $n = 3$, EM placebo: $n = 2$, and EM probiotics: $n = 1$. Therefore, this study included a total of 96 calves (LM placebo [$n = 23$], LM probiotics [$n = 25$], EM placebo [$n = 21$], and EM probiotics [$n = 27$]).

Solid feed and lying behaviors. Calf starter was provided to calves in a group bunk so individual feed intakes could not be recorded. Therefore, a subset of calves ($n = 16$; 4 EM probiotic, 4 EM placebo, 4 LM probiotic, and 4 LM placebo) born in June 2014 were observed using video for number of visits to the calf starter feed bunk (no./d) and total time spent feeding at the calf starter feed bunk (min/d), and total time spent lying (h/d). Power analysis (following Hintze, 2008) calculations found that this sample was adequate to detect a change in 20 min/d in feeding behavior between treatments with 80% power and 0.05 α . Estimates of variation for feeding behavior were based on the reported values in (Borderas et al., 2009). These feeding behaviors have been associated with DM intake (Kayser and Hill, 2013). Enrollment criteria required successful passive transfer (a serum BRIX score greater than 8.4%; Deelen et al., 2014). One infrared camera (Axis M1054 PoE cameras, Noldus Information Technology, Lund, Sweden) per pen recorded all behaviors, giving a complete view of the calf starter bunk and the automated milk feeder. Calf identification was performed based on coat pattern, leg bands, and color-coded collars. Each focal calf was continually observed and behaviors were recorded using Observer XT 11.5 (Noldus Information Technology) over a 24-h period, twice weekly until post-weaning, with 1 observation post-weaning. The start of a visit to the calf starter feed bunk was defined as the focal calf lowering its head into the calf starter bunk for longer than 2 s. The visit ended when any one of the following occurred: the focal calf lifted its head from the bunk for greater than 5 frame seconds; the focal calf turned its head away from the bunk; or the focal calf engaged in physical contact with another calf. Observers achieved a 95% inter- and intra-observer reliability with a single main observer as a baseline.

Total time lying was recorded for these focal heifers using an activity monitor (Hobo Pendant G Logger, Onset Corporation, Bourne, MA, USA) from an initial age of 3 ± 1 d (mean $\pm$ SD) until the last observation at 63 ± 3 d. Activity monitors were attached to the metacarpal of the right rear leg and were rotated to the left leg every 2 wk to prevent soreness. Lying time (h/d) was recorded at 1-min intervals and summarized in daily total time following Ledgerwood et al. (2010) and Bonk et al. (2013). The first 2 wk after enrollment in the feeder was considered an acclimation period when calves were learning how to use the feeder. The 2 periods of interest were from day 16 to day 37 during which peak milk allowance was offered for each milk

feeding strategy, and from day 48 to 4 d post-weaning, which captured activity during weaning.

Statistical Analysis

All analyses were performed in SAS statistical software v. 9.3 (SAS Institute Inc., Cary, NC, USA). Initially, descriptive statistics and residual diagnostics assessed the relationship with all outcomes reported at the univariable level, using a cutoff of $P < 0.20$ for inclusion in the model. Model fit was assessed for all outcome variables. Covariates were assessed separately for their relationship with the following dependent variables: ADG, height, milk intake, drinking speed, and calf behavior (drinking speed, calf starter visits, total time spent eating calf starter, and total time lying). The covariates that were assessed were achievement of passive transfer, dystocia, dam lactation, calf vitality score, calving location, initial weight, presence of BRD on a health exam, time of year the calf was enrolled (month), and whether or not a calf was a twin. Since experimental design anticipated a time interaction, study treatment interactions were assessed in each model. Since the effect of treatment was expected to influence growth (weight, ADG, and height models), differently based on time of milk offering, the experimental period was divided into 3 periods: from day 1 on the automated feeder to day 28 (Period 1), from day 29 to day 53 (Period 2), and the week post-weaning from day 60 to day 67 ± 2 d of age (Period 3). All models controlled for calf as the subject and week was the repeated measure. All variables included in the final models were achieved using backwards step-wise removal.

Milk intake. Daily milk intake (L/d) was analyzed using a linear mixed model controlling for month, study treatments, initial weight, and age of introduction to the feeder as fixed effects. Due to the differences in the experimental design of the milk feeding strategies, non-normality of the milk intake data and high leverage observations of daily milk intake was ranked by individual calf into 8 frames (to represent weeks every 6.7 d) using a Wilcoxon Rank Sum Test. The ranked data were assessed for statistical significance using a linear mixed model (Proc Mixed). The least-square mean (LSM) and standard error of the means (SEM) for milk intake were generated from non-transformed data. These models were repeated to look at the overall milk intake for the duration of the 53 feeder days, using the LSM and SEM for average daily milk intake (L/d).

Growth. For ADG, covariates in the linear mixed model were age and fixed effects included initial weight, month, clinical BRD on health exam, and treatment effects. For weight, covariates in the linear mixed model were age and fixed effects included initial weight, month, clinical BRD on a health exam, and treatment effects. For height, covariates in the linear mixed model were age and fixed effects included initial height and study treatment.

Feeding behavior. For drinking speed (L/s/d) recorded by the automated feeder, a linear mixed model controlled for treatment effects and for day enrolled on the feeder as a fixed effect. Due to equipment errors with the automated milk feeder computer, 1.4% (66/4868) of individual observations were excluded from the data set.

For calf starter visits (no./d/wk), covariates in the linear mixed model were fixed effects of initial enrollment weight and treatment effects. For the first 2 wk, calves visited the calf starter bunk infrequently, and due to low activity (less than 5 min/d) these were excluded.

For total time at the calf starter feeder (min/d/wk), data were not normally distributed so a log-transformation with a correction factor of 0.5 was applied. The linear mixed model of total time spent eating calf starter was controlled for fixed effect of initial enrollment weight, treatment effects, and week. Data were back transformed to obtain the geometric mean and CIs for the total time spent eating calf starter.

Lying behavior. The total time lying was performed in 2 models. The model for high milk offerings, feeder day 16 to feeder day 37 controlled for treatment effects, week, and the age enrolled on the feeder as a fixed effect. The second model assessed for the effect of study treatment on lying during weaning. The total time lying linear mixed model for weaning controlled for week and study treatment.

RESULTS

Milk Intake

There was an interaction between feeder week and milk feeding strategy ($P < 0.001$; Figure 2). Milk intake was significantly different between milk feeding strategies for week 1, 4, 5, 6, and 7

($P < 0.001$; Figure 2). Probiotics did not affect milk intake during the experimental period ($P = 0.22$; Figure 2).

For total milk intake across the experimental period, LM calves consumed significantly more milk than EM calves (431.84 ± 33.02 liters vs. 378.64 ± 34.21 liters; $P < 0.001$; LSM $\pm$ SEM). For milk intake across the experiment (the 53 feeder days), probiotics and placebo were not significantly different (408.91 ± 39.27 liters vs. 401.16 ± 046.97 liters; $P = 0.96$).

Performance

Birth weights of calves were similar across study treatments (39.79 ± 5.27 kg; mean $\pm$ SD). Calves weighed 86.03 ± 2.47 kg at weaning, and 92.81 ± 2.48 kg at 1 wk post-weaning. Probiotics affected ADG for Period 1, and probiotic calves had greater ADG than placebo calves (Figure 3; $P = 0.01$). Milk feeding strategy affected ADG for Period 3, and EM calves had greater ADG than LM calves ($P = 0.04$).

Milk feeding strategy affected weights at Period 2, with weights for the LM calves being higher than weights of the EM calves ($P = 0.01$); probiotics affected weights at Period 1, with probiotic calves weighing more than placebo calves (Figure 4; $P = 0.03$). For Period 2, probiotic calves tended to weigh more than placebo calves (76.43 ± 0.90 kg vs. 74.20 ± 0.91 kg; $P = 0.06$). Weights at 1-week post-weaning were not different across treatments, milk feeding strategy ($P = 0.65$), and probiotics ($P = 0.22$).

For height, calves at 3 ± 2 d were 76.38 ± 2.25 (LSM $\pm$ SEM) cm tall and were balanced across study treatments. For Period 1, calves were 82.49 ± 2.22 cm tall. For Period 2, calves were 88.87 ± 2.29 cm tall. For Period 3, calves were 92.33 ± 2.27 cm tall. Milk feeding strategy and probiotics did not affect height ($P = 0.70$ and $P = 0.98$, respectively).

Health

There was an equal representation of failure of passive transfer, incidence of BRD, and incidence of diarrhea in all study treatment groups. Successful passive transfer occurred in 92% (88/96) of the population and total serum BRIX ranged from 8.0% to 13.0%. Ninety percent (86/96) of calves were diagnosed with BRD, 23% (22/96) had 1 case, 44% (42/96) had 2 cases, and 23% (22/96) were chronic. The incidence of diarrhea was 82% (79/96), with 30% (29/96) of calves having 1 case, 34% (33/96) having 2 cases, and 18% (17/96) having 3 cases. Six calves received additional oral electrolytes (EM placebo [$n = 3$], LM placebo [$n = 2$], and LM probiotics [$n = 1$]). Since this study was not designed to assess the effect of probiotics on the odds of having an additional case of diarrhea, we did not have statistical power to test for this effect.

Feeding Behavior

Drinking speed. Over the entire milk feeding period, the drinking speed was 0.73 ± 0.50 L/min. There was an interaction between milk feeding strategy and week on the feeder ($P < 0.001$; Figure 5).

Figure 2. Least square average daily milk intake (L/d) by week, for Holstein heifers ($n = 96$) fed either a milk feeding strategy that peak at 21 or 28 d¹ and lactic acid probiotic, or placebo². ¹Treatments: EM = early milk feeding strategy feeder day 1 offers 11 L/d and milk peaks at 15 L/d at 21 feeder days; LM = late milk feeding strategy feeder day 1 offers 7 L/d and milk peaks at 13 L/d at 28 feeder day. ²Probiotic = active lactic acid strain probiotic fed to calves 2 $\times$ /d while on the feeder.

Figure 3. Least square average daily gain (kg/d) reported by period¹, for Holstein heifers ($n = 96$) fed either a milk feeding strategy that peak at 21 or 28 d² and lactic acid probiotic, or placebo³. *Significant differences by period for Probiotics Period 1 ($P = 0.01$), and Milk Feeding Strategy in Period 3 ($P = 0.04$); Milk feeding strategy*Probiotic was not significant ($P = 0.25$). ¹Study Period 1: enrollment on automated feeder (7 ± 2 d) to feeder day 28. Period 2: feeder day 29 to day 53. Period 3: the week post-weaning. ²Treatments: EM = early milk feeding strategy feeder day 1 offers 11 L/d and milk peaks at 15 L/d at 21 feeder days; LM = late milk feeding strategy feeder day 1 offers 7 L/d and milk peaks at 13 L/d at 28 feeder days. ³Probiotic = active lactic acid strain probiotic fed to calves $2 \times /d$ while on the feeder.

Figure 4. Least square average body weight (kg) reported by period¹, for Holstein heifers ($n = 96$) fed either a milk feeding strategy that peak at 21 or 28 d² and lactic acid probiotic, or placebo³. *Significant differences by period for Probiotics in Period 1 ($P = 0.03$), and Milk Feeding Strategy in Period 2 ($P = 0.01$). ¹Study Period 1: enrollment on automated feeder (7 ± 2 d) to feeder day 28. Period 2: feeder day 29 to day 53. Period 3: the week post-weaning. ²Treatments: EM = early milk feeding strategy feeder day 1 offers 11 L/d and milk peaks at 15 L/d at 21 feeder days; LM = late milk feeding strategy feeder day 1 offers 7 L/d and milk peaks at 13 L/d at 28 feeder days. ³Probiotic = active lactic acid strain probiotic fed to calves $2 \times /d$ while on the feeder.

For week 2 and week 3, the EM calves drank slower than LM ($P < 0.001$ and $P < 0.001$, respectively). For week 4 and week 5, the EM calves drank faster than LM calves ($P < 0.001$ and $P < 0.001$, respectively). Week 1 ($P = 0.45$), week 6 ($P = 0.25$), week 7 ($P = 0.77$), and week 8 ($P = 0.80$) were not

different. Probiotics did not affect drinking speed ($P = 0.72$).

Solid feeding behavior. For calf starter visits, probiotic and week interacted ($P = 0.01$; Table 1). Milk feeding strategy did not affect calf starter visits

Figure 5. Drinking speed (L/min) for Holstein heifers ($n = 96$) fed either a milk feeding strategy that peak at 21 or 28 d¹ and lactic acid probiotic, or placebo². ¹Treatments: EM = early milk feeding strategy feeder day 1 offers 11 L/d and milk peaks at 15 L/d at 21 feeder days; LM = late milk feeding strategy feeder day 1 offers 7 L/d and milk peaks at 13 L/d at 28 feeder days. ²Probiotic = active lactic acid strain probiotic fed to calves 2×/d while on the feeder.

($P = 0.62$). Calf starter visits did not differ between probiotics and placebo calves ($P = 0.57$).

For total time spent eating calf starter, milk feeding strategy ($P = 0.25$) and probiotics ($P = 0.65$) were not significant. Since these data were transformed, the antilog geometric means and CIs were reported. The geometric mean time at the bunk was 4.83 min/d (95% CI = 1.23 to 1.60) for week 3, 7.45 min/d (95% CI = 1.83 to 2.89) for week 4, 11.78 min/d (95% CI = 2.85 to 3.72) for week 5, 30.10 min/d (95% CI = 7.07 to 9.19) for week 6, 33.42 min/d (95% CI = 7.83 to 10.17) for week 7, and 51.79 min/d (95% CI = 12.06 to 15.68) for the 4 d post-weaning.

Total time lying. During the milk-feeding period (feeder day 16 to feeder day 37), the milk feeding strategy interacted with the probiotics ($P = 0.04$). While on milk, the EM placebo total time lying was 16.24 ± 0.34 h/d, which was significantly lower than both probiotic groups (EM probiotics 17.86 ± 0.26 h/d, $P = 0.01$; LM probiotics 17.42 ± 0.29 h/d, $P = 0.03$). The EM placebo calves tended to have lower lying time compared to LM placebo calves (17.26 ± 0.26 h/d, $P = 0.07$). However, during the milk-feeding period, lying time was not different between milk feeding strategies for those calves receiving probiotics ($P = 0.30$).

During and after weaning, total lying time was affected by an interaction between probiotics and milk feeding strategy ($P = 0.01$). The LM probiotic group lying time (15.48 ± 0.32 h/d) was lower than LM placebo (16.65 ± 0.32 h/d, $P = 0.02$) and EM probiotics (16.86 ± 0.32 h/d, $P < 0.01$), but the LM probiotic group lying time was not different than

EM placebo calves (16.28 ± 0.32 h/d, $P = 0.15$). The LM placebo and EM probiotics group lying times were not different ($P = 0.90$).

DISCUSSION

This study investigated the changes in growth and feeding behavior of calves depending on the timing of peak milk allowance and the supplementation of a lactic acid bacteria-based probiotic program, while using an automated feeding system. Researchers have demonstrated that *ad libitum* feeding of milk to calves in automated milk feeding systems leads to higher growth than limit-fed calves when gradually weaned, but calf starter intake after weaning was lower than limit-fed calves (Miller-Cushon et al., 2013). In a commercial automated milk system setting, there may be interest in offering high levels of milk and a lactic acid bacterium-based probiotic program to improve growth without negating early calf starter intake. We found that when calves were offered a peak milk allowance at 5 wk of age (LM), they had greater total milk intake and improved weights at 7 wk of age when compared to calves offered peak milk allowance a week sooner (4 wk of age); however, these differences were not statistically significant at 1 wk post-weaning (8 wk).

Similarly, for a lactic acid bacteria-based program, probiotic calves had higher body weights during Period 1 than the placebo, but growth differences disappeared by 1 wk post-weaning. Interestingly, neither probiotics nor milk feeding strategy affected total time at the calf starter feed bunk; probiotic calves just visited the calf

Table 1. Calf starter visits (bouts per day) by week for dairy calves ($n = 16$) fed either a milk feeding strategy that peaked at 21 or 28 d and lactic acid probiotic, or placebo during 53 d

Feeder week	Milk strategy ¹			Active lactic acid feeding ²		
	EM	LM	Milk strategy, P -value	Probiotic	Placebo	Lactic acid feeding, P -value
3	8.5 $\pm$ 2.8	8.0 $\pm$ 2.8	0.70	7.4 $\pm$ 2.8	9.5 $\pm$ 2.8	0.22
4	9.3 $\pm$ 2.8	8.0 $\pm$ 2.8	0.33	7.8 $\pm$ 2.8	10.3 $\pm$ 2.8	0.21
5	12.5 $\pm$ 3.0	12.0 $\pm$ 3.0	0.73	11.9 $\pm$ 3.0	13.0 $\pm$ 3.0	0.27
6	18.2 $\pm$ 2.8	17.5 $\pm$ 2.8	0.43	18.8 $\pm$ 2.8	17.0 $\pm$ 2.8	0.33
7	22.8 $\pm$ 2.9	22.5 $\pm$ 2.9	0.84	22.9 $\pm$ 2.9	23.3 $\pm$ 2.9	0.46
8	31.7 $\pm$ 2.8 ^a	28.4 $\pm$ 2.8 ^a	0.20	37.8 $\pm$ 2.8 ^b	23.2 $\pm$ 2.8 ^c	<0.01

^{a-c}Least square means within a row with different superscripts differ ($P < 0.01$).

¹Treatments: EM = early milk feeding strategy feeder day 1 offers 11 L/d and milk peaks at 15 L/d at 21 feeder days; LM = late milk feeding strategy feeder day 1 offers 7 L/d and milk peaks at 13 L/d at 28 feeder days.

²Probiotic = active lactic acid strain probiotic fed to calves 2 $\times$ /d while on the feeder.

starter feed bunk in more frequent visits. In some instances, solid feeding time is also associated with DM intake, and explained variation in residual feed intakes in feeder bulls (Kayser and Hill, 2013), but not in dairy calves. Therefore, although total feed intake cannot be recorded per animal, feeding behavior at the feed bunk can provide insight into the meal patterns of dairy calves and their feeding intake. Further research should investigate the relationship between time spent at the feed bunk and DM intake in dairy calves. Since total time at the calf starter feed bunk was not different between milk feeding strategies, both milk feeding strategies effectively transition calves onto solid feed. In fact, as milk offerings decreased, total time at calf starter feed bunk increased for both milk feeding strategies, with no differences (12 min at week 5 vs. 33 min at week 7 and 52 min after weaning). However, offering milk a week later (LM) leads to much more milk consumption for the same growth.

Milk Intake and Solid Feed

EM calves did not consume as much milk (379 liters) as LM calves (472 liters), likely due to differences in the timing when peak milk allowance was offered. There is a wealth of literature describing the benefits of providing high milk allowances early in life, including higher weight gains and reduced behavioral signs of hunger (Vieira et al., 2008; Rosenberger et al., 2017) as well as more frequent and more even diurnal patterns of feeding activity (Miller-Cushon et al., 2013) compared to calves receiving lower milk allowances. In addition, there is evidence that offering more milk to calves (compared to lower milk allowances) has long-term benefits including an association with greater mammary development (Geiger et al., 2016) and increased milk production observed in the first

lactation (Gelsinger et al., 2016). For this study, both calf groups consumed more milk than is typically offered to calves fed restricted milk allowances of 6 L/d (319 liters milk allowance total). However, our milk feeding strategy was different than other studies which offered constant daily levels of milk (see review by Miller-Cushon and DeVries, 2015) in that our calves were restricted on milk for only parts of the pre-weaning period. In fact, drinking speed in this study was highest during the weeks when milk was most restricted (i.e., week 2 to 3 for LM; week 4 to 5 for EM). This suggests that drinking speed may be associated with hunger. However, our milk feeding strategies used timing as an element of milk restriction prior to weaning. We hypothesize that our EM calves consumed less milk than LM calves due to meal size restriction during the first weeks on the feeder. This is supported by Jensen (2009), who observed a calf's milk meal pattern is affected by settings of the automated feeder.

Few studies have investigated how incrementally increasing the daily milk allowance in the first weeks of life, and the subsequent timing of maximum peak milk allowance, impact performance and behavior in calves. Also, calves were also fed in only 2 meals in most studies making comparisons inappropriate (i.e., Davis Rincker et al., 2011; Hill et al., 2016). For example, calves have been fed increasing levels of total milk solids throughout the pre-weaning period (Hill et al., 2016). In other studies, calves were fed according to an adjusted weekly body weight (Davis Rincker et al., 2011), but they were fed in only 2 meals. Studies where calves were offered similar milk levels in multiple meals on automated milk feeders (Rosenberger et al., 2017) are also difficult to reference to our study as we did not offer consistent daily maximum intakes. These studies are different from our research, in that we used an automated milk feeding schedule

to provide high milk allowances in multiple meals to the calf. Making comparisons in growth to other automated milk feeder studies is difficult because we changed the daily amount of milk offered to the calf. However, we cannot distinguish whether differences in milk intake are due to offering more milk earlier or due to the timing of peak allowance.

When calves were provided probiotics daily from birth to weaning, there was no effect on milk intake. Our results are supported by Geiger et al. (2014) who found that a similar probiotic strain did not affect milk intake for individually housed calves that were fed a traditional milk replacer (22% crude protein, 20% fat) or an accelerated milk replacer (27% crude protein, 10% fat) (Geiger et al., 2014). However, our results are different from Soto et al. (2014), where calves were offered 6 L/d but consumed, on average, less than 4 L/d in an automated feeding system; at these restricted levels of milk, calves are known to experience hunger, which is expressed in behaviors such as vocalizations, increased visits to the milk teat (Vieira et al., 2008), and in limited growth (Roth et al., 2009), when compared to calves that are fed higher levels of milk. Since our study offered a minimum of 7 L/d for LM calves and 11 L/d for EM calves when calves were allocated to the automated milk feeder, the lack of restricted nutritional intake in the first weeks of life may have limited the available benefits from the probiotics, at least for measures of milk intake, and feeding behaviors. However, this does not necessarily mean there are no benefits of feeding probiotics to calves receiving higher milk allowances; for instance, probiotics might encourage calves to visit the calf starter bunk in smaller meals rather than eating calf starter in larger meals after weaning as did calves receiving the placebo. Speculatively, our calves may have benefited post-weaning from probiotic supplementation during the milk feeding period since probiotics stabilize intestinal microflora post-weaning (Bridget et al., 2018). It is possible that additional visits to solid feed were expressed in a more stable intestinal environment, though we cannot confirm this in our study. The influence of probiotics on intestinal microbes after weaning is a potential future direction for probiotic research regarding calves fed higher levels of milk in automated milk feeding systems.

Performance

In this study, ADG and weights were not different between milk feeding strategies from feeder enrollment to peak milk offering for LM at feeder day 28 (Period

1). This was likely due to milk intake only being different during week 1 and week 4. Milk intake was not different between groups, even when early calves were offered a bigger milk allowance. However, despite a lack of difference in milk intake from feeder enrollment to feeder day 28 (Period 1), ADG was higher for probiotic calves over placebo. This is likely a function of competitive exclusion, as lactic acid probiotics also influences neutrophil-to-lymphocyte ratios, which is associated with increased growth in calves without influencing milk or solid feed intake (Noori et al., 2016). Though this study did not measure immune function, probiotic calves in this study did reflect higher growth without influencing milk intake. However, a meta-analysis found that lactic acid-based probiotics programs are not associated with improving growth when fed whole milk (Frizzo et al., 2011). Since our probiotic calves did not weigh differently by 1 wk post-weaning than other study treatments, we can conclude that our calves had limited benefits from probiotics in growth. However, our study is different from this meta-analysis because our calves were fed a blend of lactic acid bacteria and yeast. Our probiotic blend contained the yeast strain *S. cerevisiae*. According to an extensive review, *S. cerevisiae* yeast was associated with improving growth without increasing intakes, but it cautioned to best benefit calves under intense disease pressures (Alugongo et al., 2017). This finding supports our study, where calves on the probiotic blend reflected better growth than calves on the placebo without demonstrating differences in milk intake. Although we cannot separate the effects between microbial strands, probiotics likely operated on improving ADG in Period 1 through competitive exclusion.

No differences in ADG between milk feeding strategies and probiotics in the first week post-weaning was found. Other studies on automated milk feeding system found that calves on higher milk allotments have much higher ADG than calves allotted 6 L/d when compared on a mega calories per kilogram basis (Rosenberger et al., 2017). It is possible that EM calves benefited from epigenetic programming, which is a hypothesis that suggests early life nutrition alters hormonal mechanisms, affecting gene expression and changing growth (Kertz et al., 2017). Indeed, this is a potential explanation for why calves had promoted mammary tissue development when calves were fed more milk-based protein (Geiger et al., 2016), and why others have seen earlier puberty in calves fed more milk (Davis Rincker et al., 2011). However, more research is needed to understand these mechanisms in neonatal dairy calves to investigate this hypothesis.

During the experiment, we found high disease, though disease rates did not differ across study treatments. For example, an epidemiological study which followed 17 dairies using automated feeding systems for a year in Canada reported a prevalence of diarrhea of 23% and BRD was 17% (Medrano-Galarza et al., 2017). Disease does vary by study and by farm. For example, prevalence of BRD was 57% for a behavioral study in Wisconsin, United States, which observed calves fed acidified milk ad libitum from the same nipple (Cramer and Stanton, 2015). Speculatively, we hypothesized that EM calves may have not consumed their maximum allotted milk when compared to LM calves during week 2 and week 3 due to high neonatal diarrhea affecting all calves during EM peak milk offering. This is supported as calves are most susceptible to neonatal diarrhea between 2 and 3 wk of age (Cho and Yoon, 2014). Neonatal diarrhea was also associated with lower milk intake for an epidemiological study following 10 dairies using automated feeding systems for 8 mo in the United States (Knauer et al., 2017). In addition, our probiotic calves had higher ADG during this time. We speculate competitive exclusion may have benefited these calves during disease. Competitive exclusion from probiotics was associated with improving calf growth in a meta-analysis for calves fed whole milk (Frizzo et al., 2011), and in veal calves under high disease pressures (Timmerman et al., 2005). However, since we had similar rates of diarrhea across study treatments we can only speculate. We also controlled for BRD, and other diseases in our growth model, therefore, we are statistically limited in our interpretation of the effects of disease on the outcomes. Future research is needed to determine the effects of probiotics and milk feeding strategies on high disease herds.

Lying Behavior

Lying time for our calves was similar to that of calves housed in groups, and both showed decreases in lying time at weaning (Eckert et al., 2015). Offering probiotics to LM calves appeared to decrease lying time when compared to other groups. Lower lying times are associated with stress, as reported by Theurer et al. (2012), who found that calves stand more when they are not provided non-steroidal anti-inflammatory drugs after dehorning. However, the adverse effect on lying time at weaning caused by probiotic use in LM calves is not well understood; however, this reduced lying time did not affect time spent at the calf starter feed bunk and did not affect growth. Future

research should investigate the interactions between probiotic use and milk feeding strategies.

CONCLUSION

In conclusion, we found that the supplementation of probiotics from birth lead to improved ADG during the first 28 d of life, and more visits to the calf starter feed bunk after weaning, but this was not reflected in higher body weights at 1 wk post-weaning. Calves receiving an early accelerated milk allowance consumed less milk in total, had improved growth during weaning, and had similar weights at 1 wk post-weaning to those calves on a late peak milk allowance program. Milk feeding strategies did not affect the duration of time that a calf spent at calf starter feed bunk or the number of times that a calf visited the calf starter feed bunk throughout the study. Probiotics had limited benefits in reducing disease incidence in a high disease prevalence herd. However, an early milk feeding strategy in a herd with high disease pressures had similar growth and solid feeding behavior in calves for less milk consumption compared to calves offered peak milk a week later on the feeder. *Conflict of interest statement.* None declared.

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